

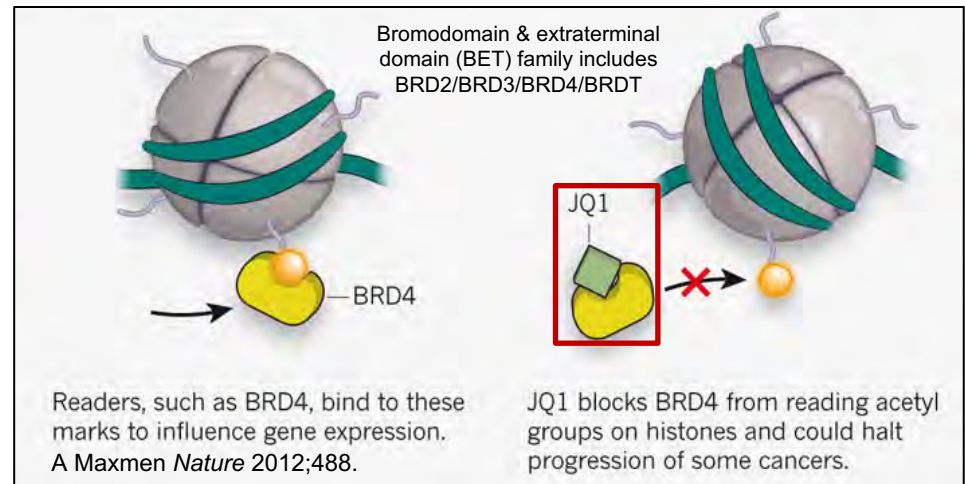
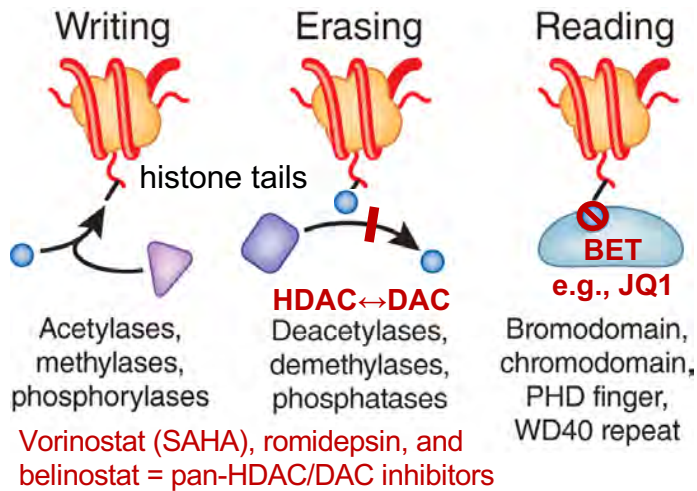
14th Annual Texas Conference on Health Disparities

Anti-Cancer Strategies Targeting Epigenetic Readers, Writers and Erasers

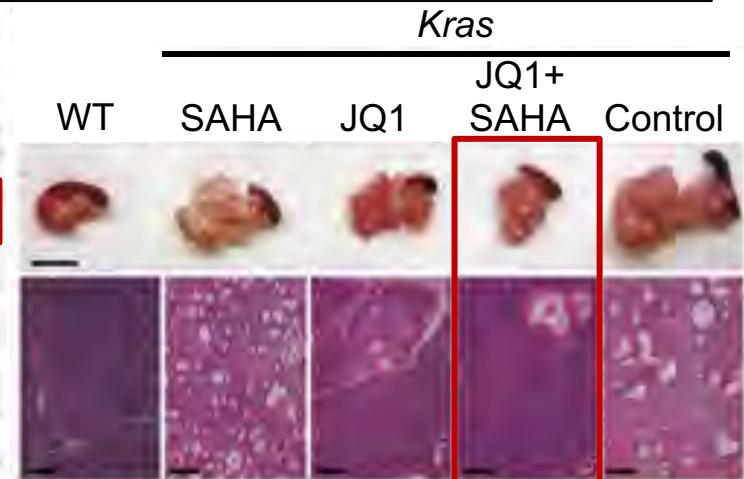
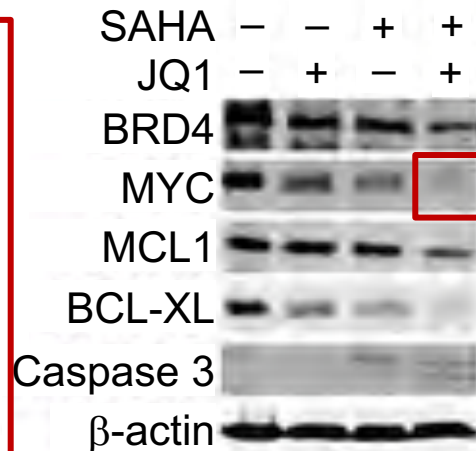
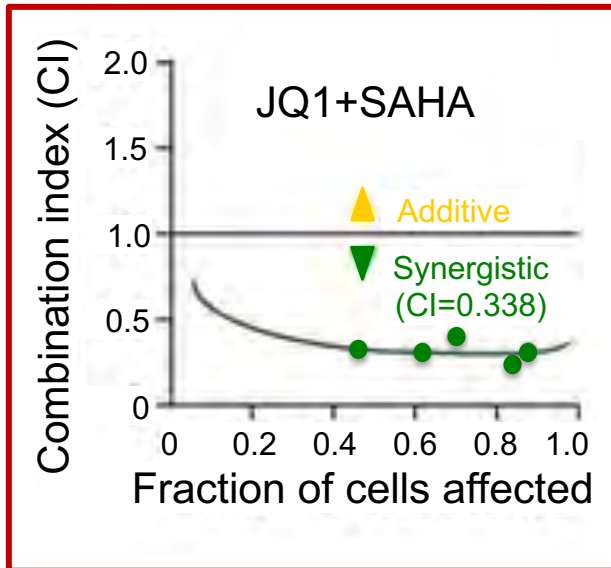
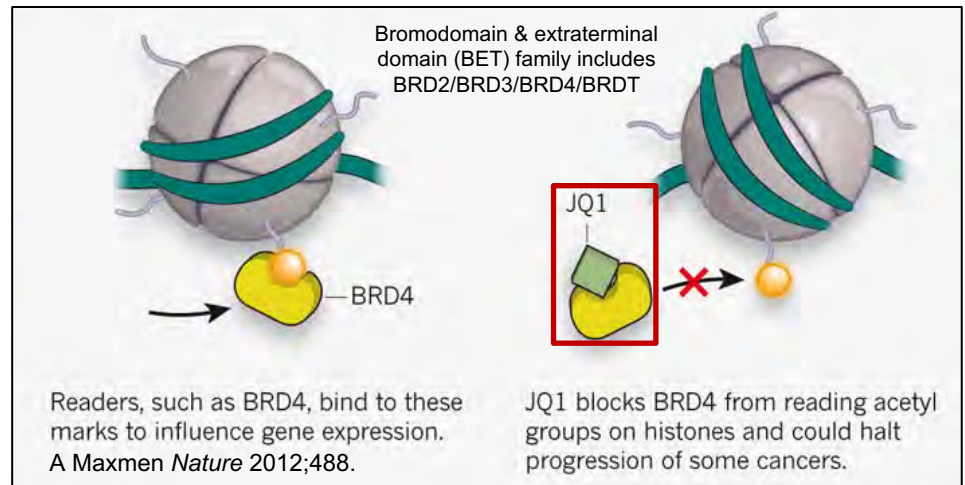
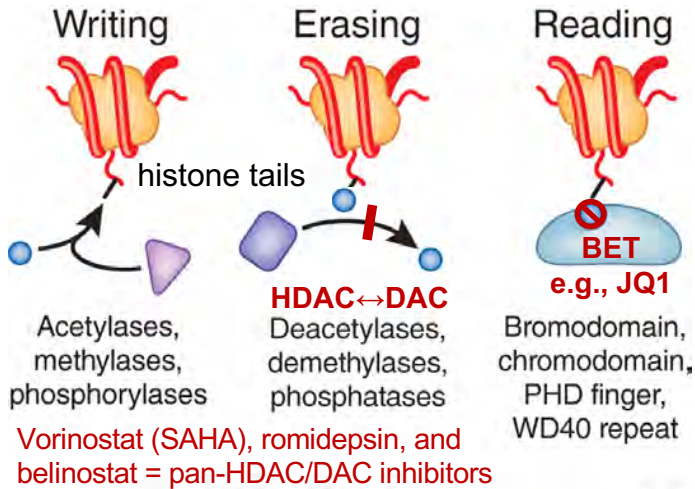
*Roderick H. Dashwood, Ph.D., FRSB, Director
Center for Epigenetics & Disease Prevention,
Texas A&M College of Medicine
Houston, Texas*



Epigenetic 'readers, writers and erasers'

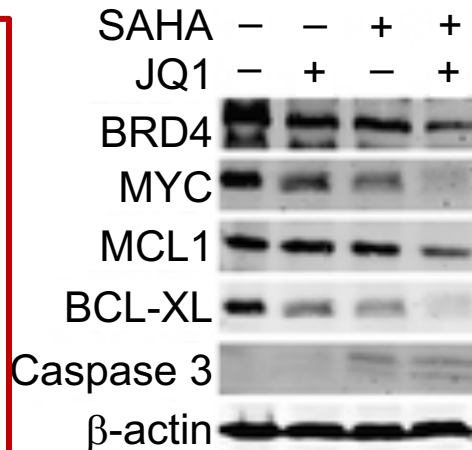
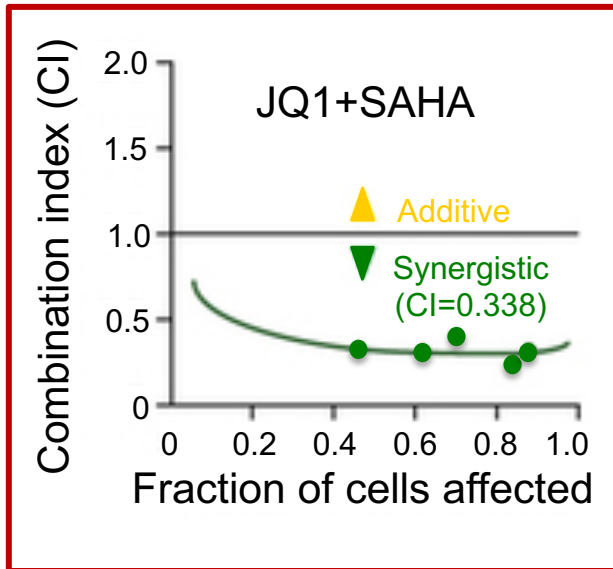
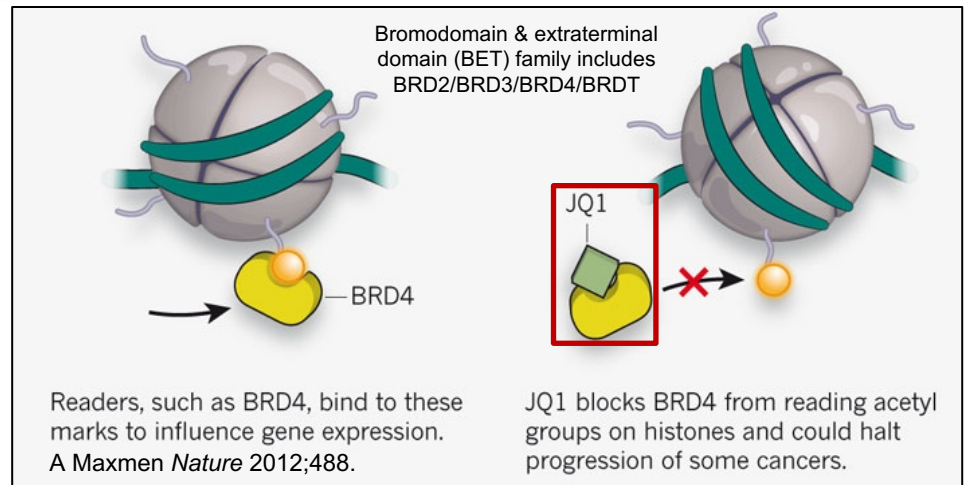
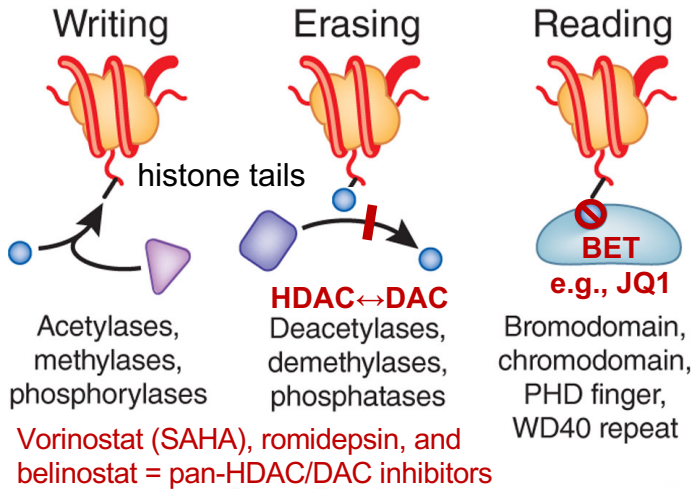


'Drugging chromatin'



Combined inhibition of BET family proteins and HDACs as a potential **epigenetics-based therapy** for pancreatic ductal adenocarcinoma. Mazur *et al.*, *Nat Med* 2015.

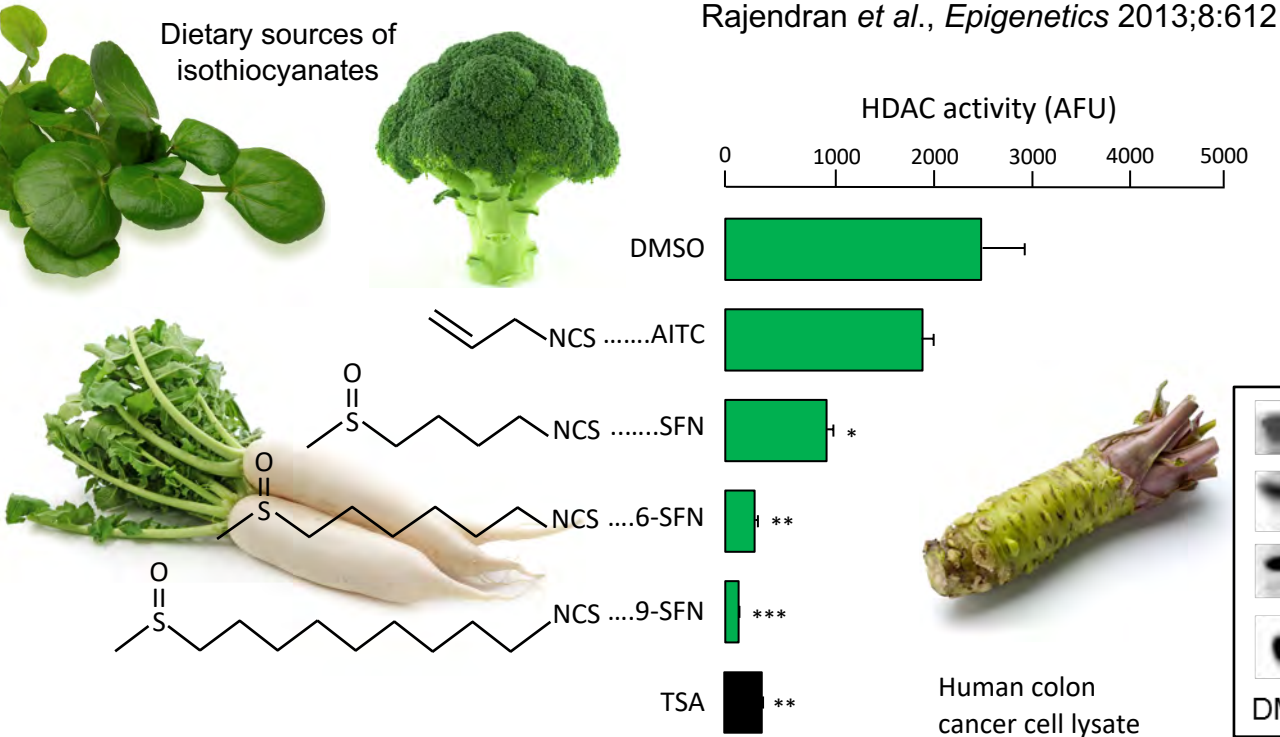
'Drugging chromatin'



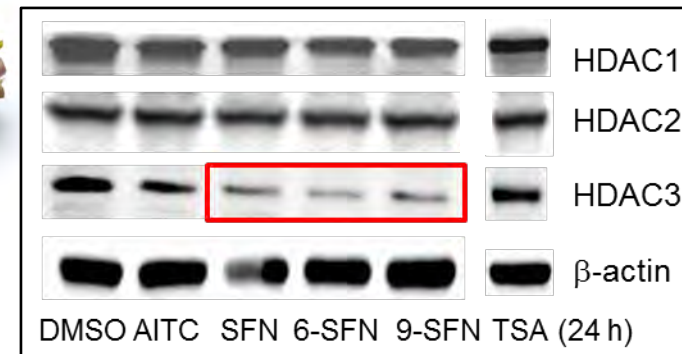
High-throughput screening in cell-based assays, >80,000 drugs/natural products → sulforaphane

HDAC3 turnover by dietary isothiocyanates

Praveen Rajendran, Ph.D.

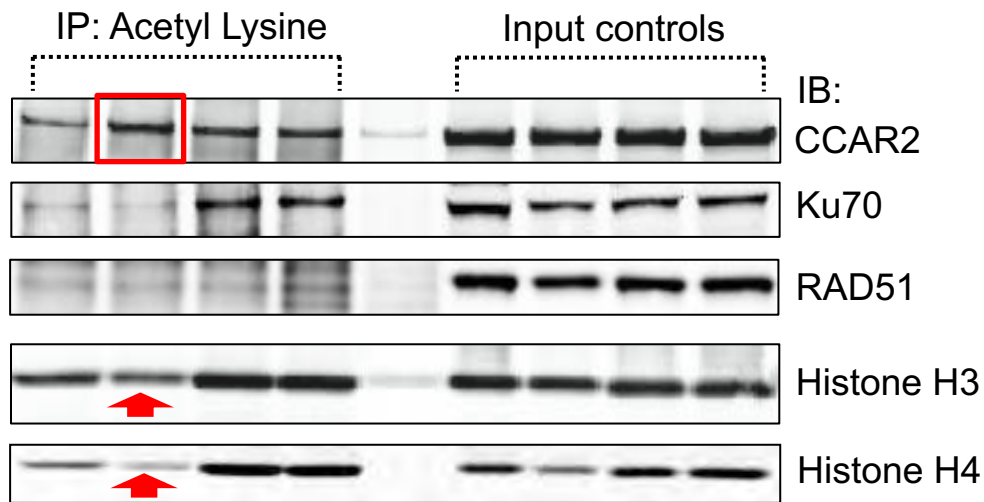


Human HCT116 colon cancer cells

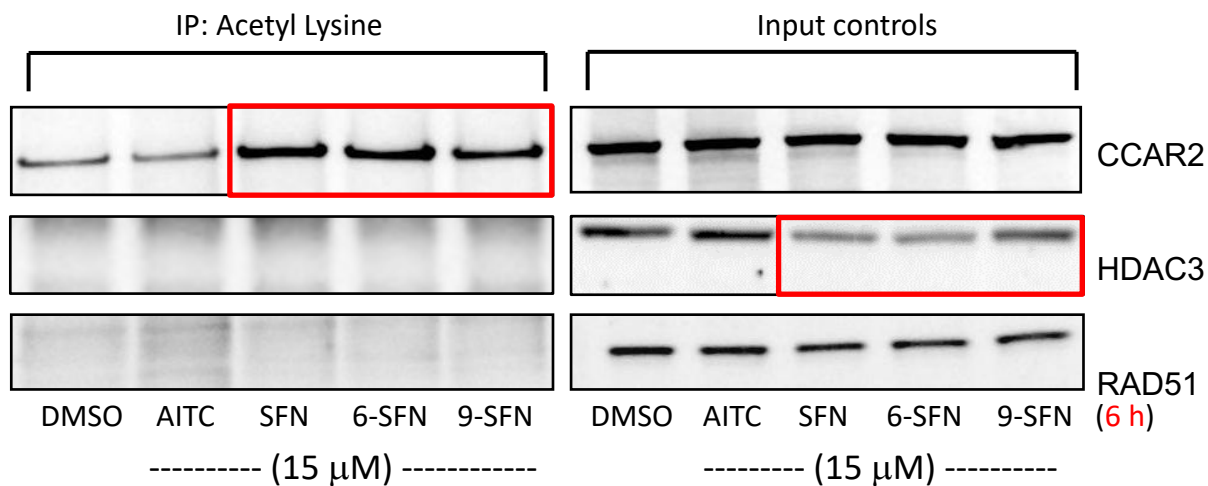
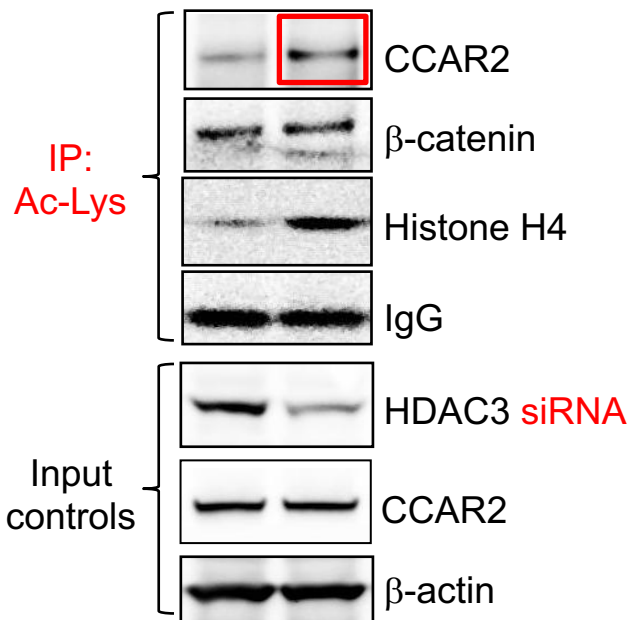
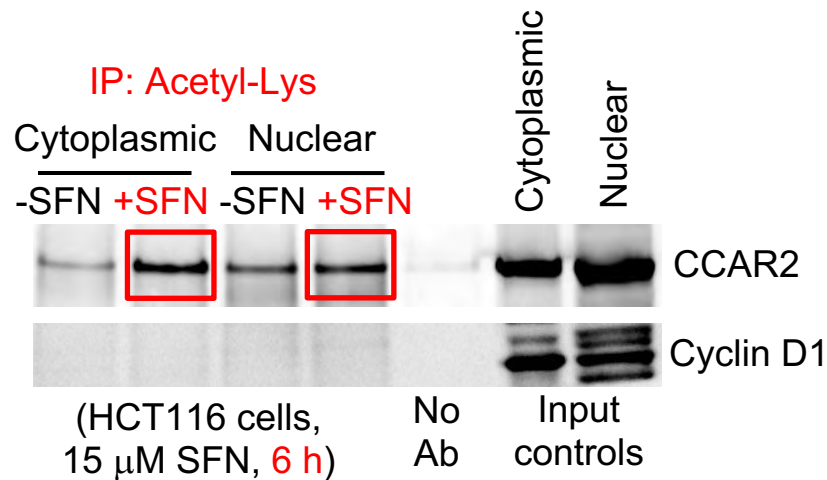


- Immunoaffinity purification using acetyl-Lys antibodies → protein mass spectrometry.
- **CCAR2 was the earliest target for acetylation by SFN in human colon cancer cells.**
- Cell Cycle and Apoptosis Regulator 2 (CCAR2) is a 'master regulator' of metabolism, aging, and cancer' EN Chini *et al.*, 2013.

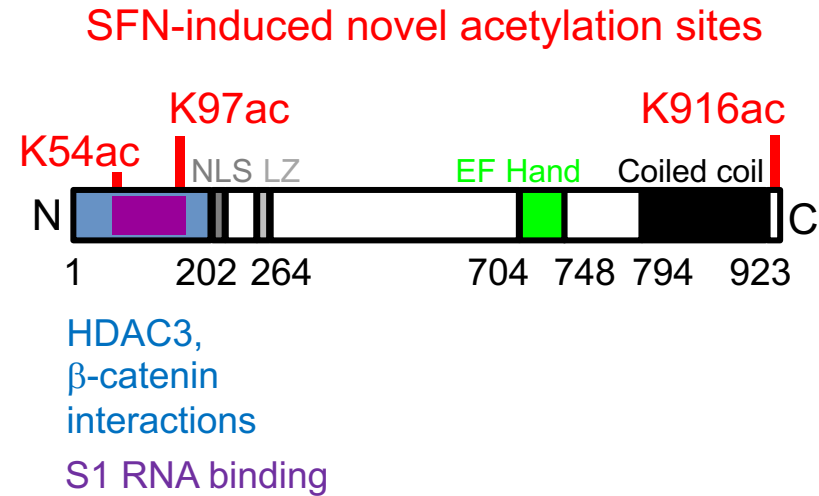
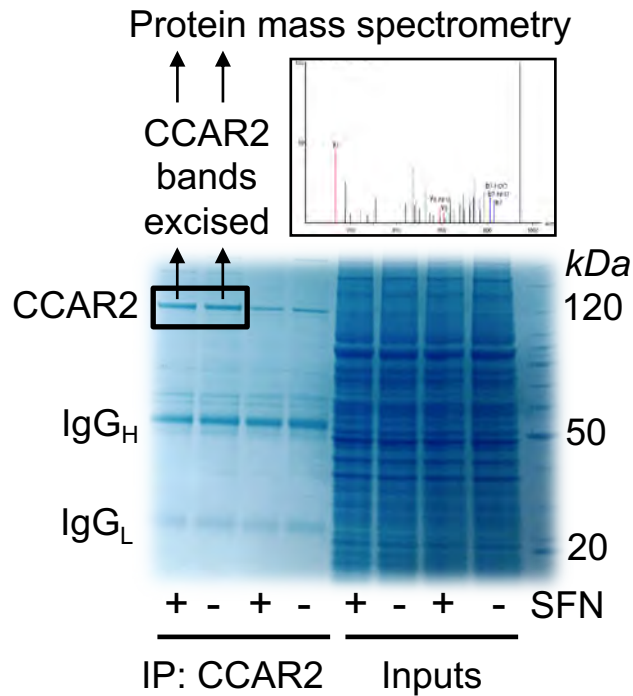
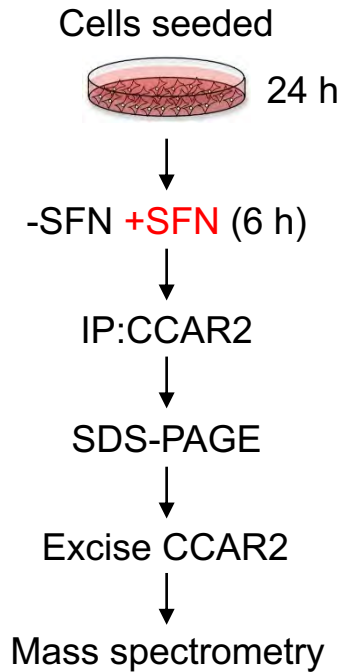
CCAR2 acetylation precedes histone acetylation



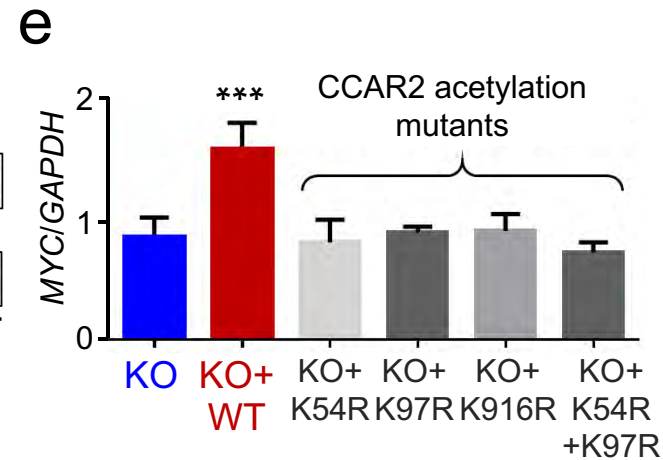
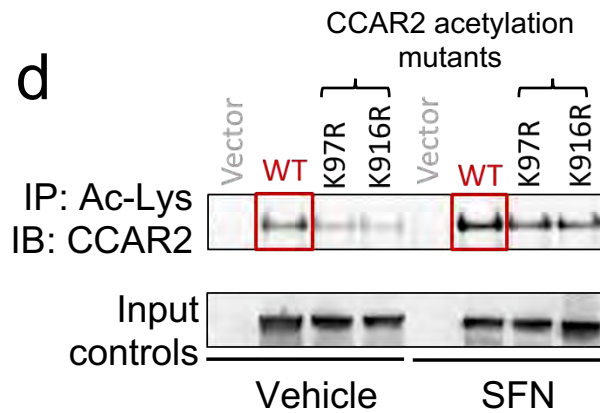
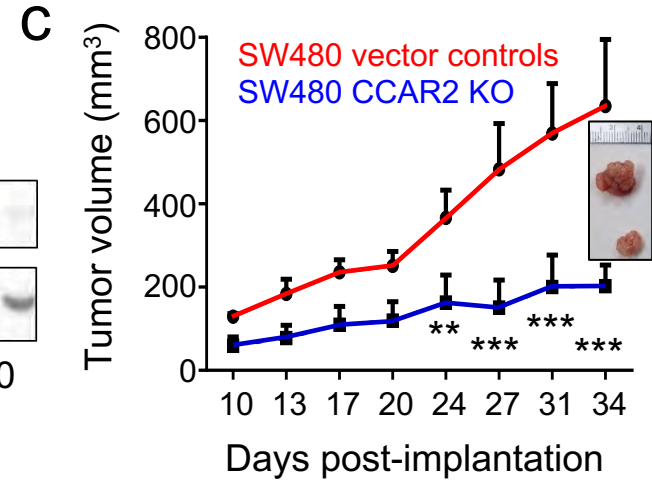
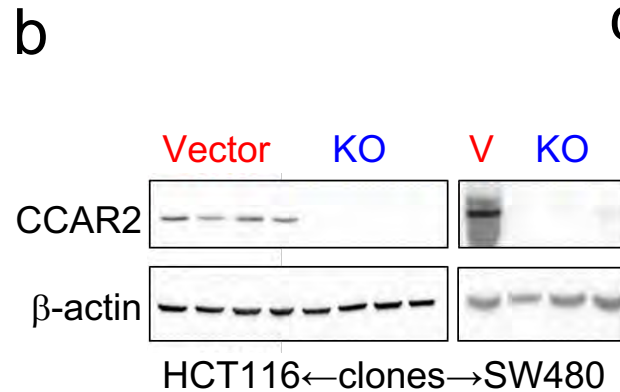
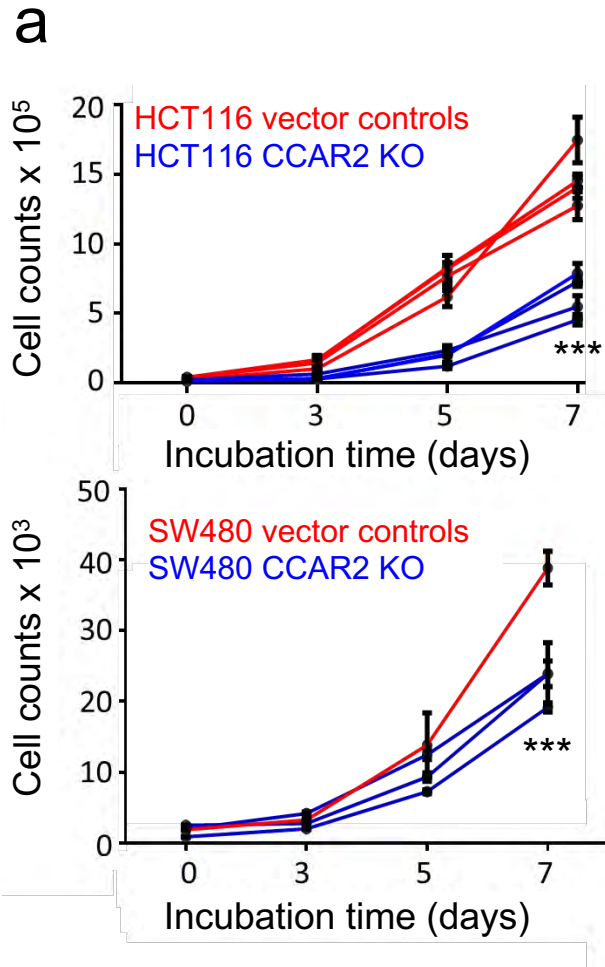
A DMSO SFN NaB TSA (6 h)



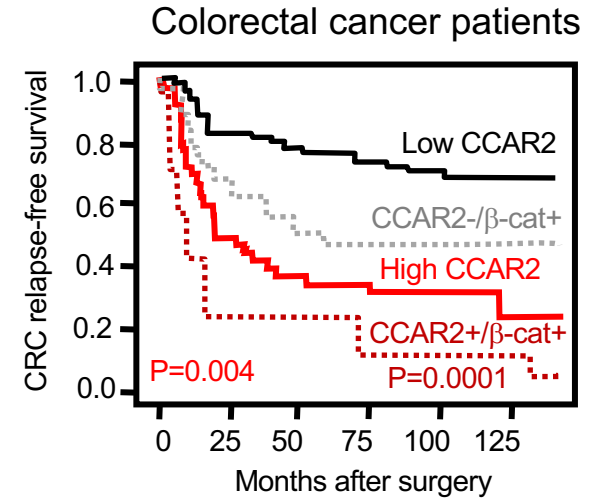
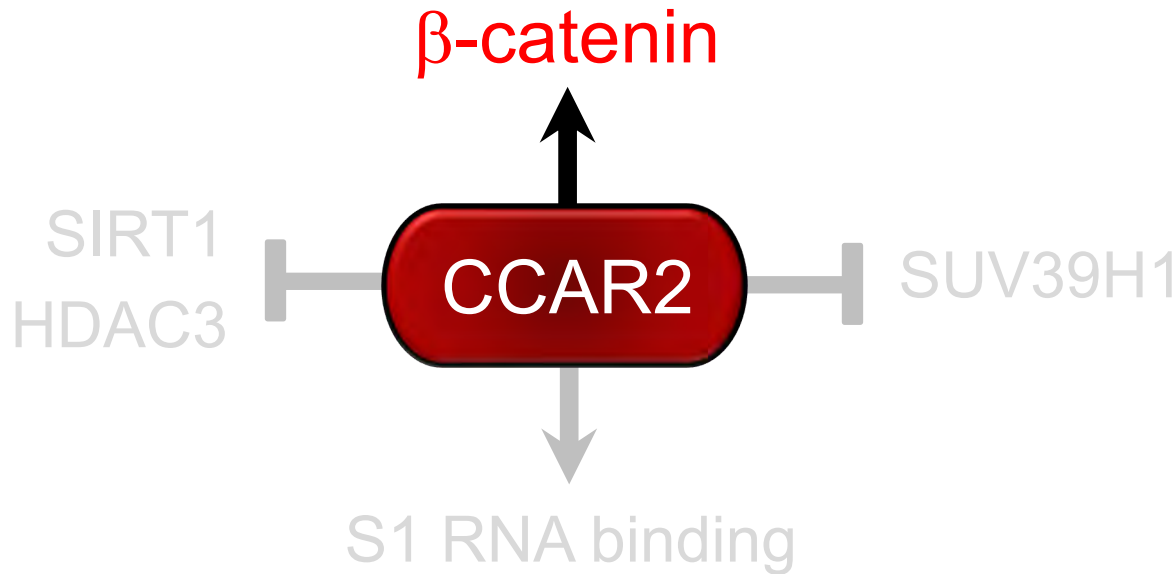
Identifying the CCAR2 acetylation sites



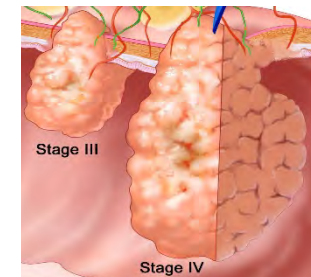
CCAR2 null cells are rescued by WT CCAR2



CCAR2 is a *coactivator* of Wnt/ β -catenin signaling

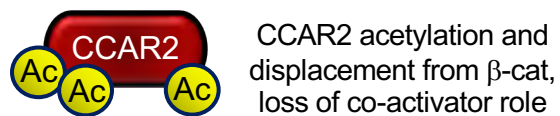
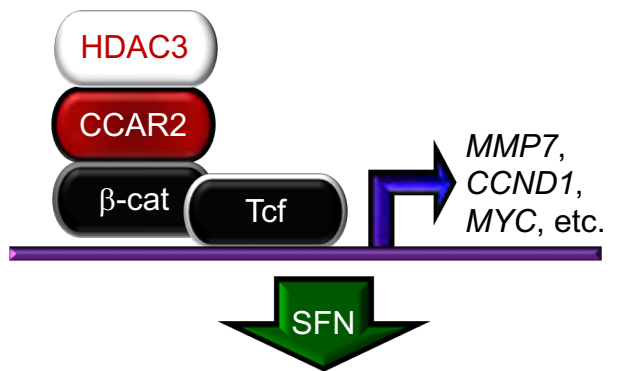


(EJ Yu *et al.*, *Oncogene* 2016)

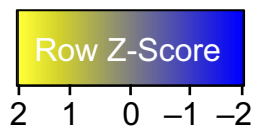
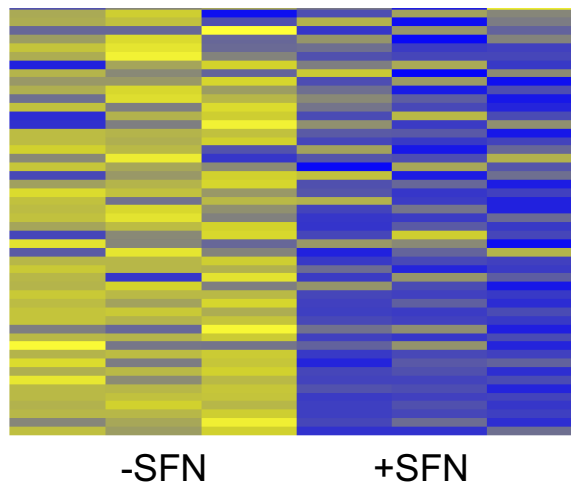
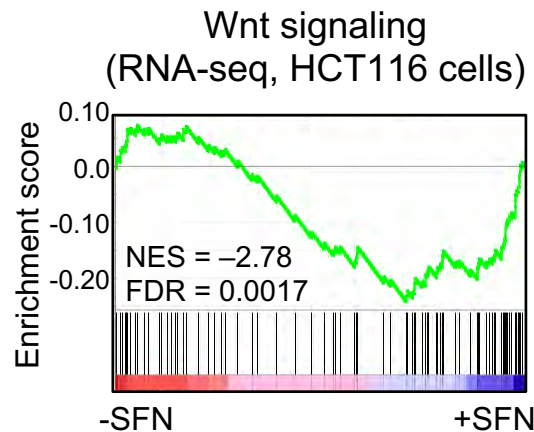


Colorectal cancer (CRC)

Wnt/ β -catenin signaling is diminished by SFN

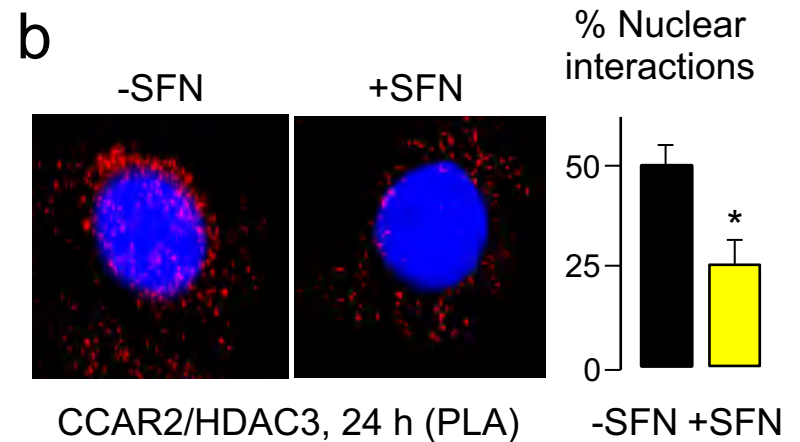
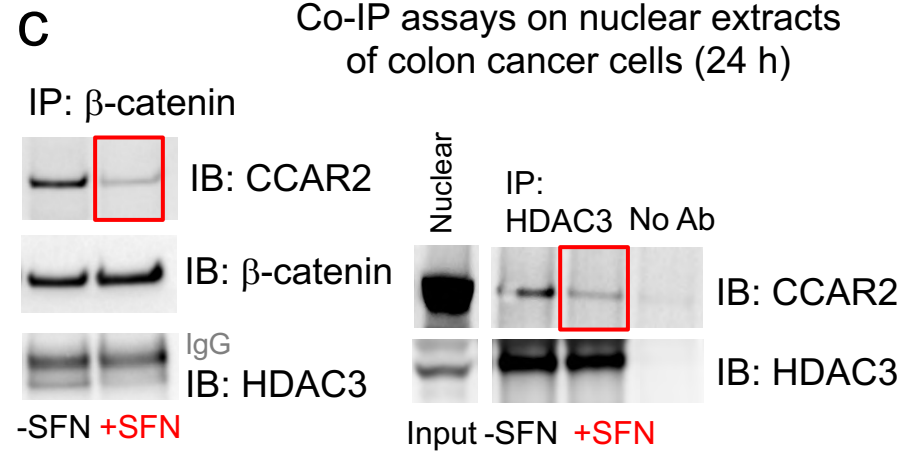
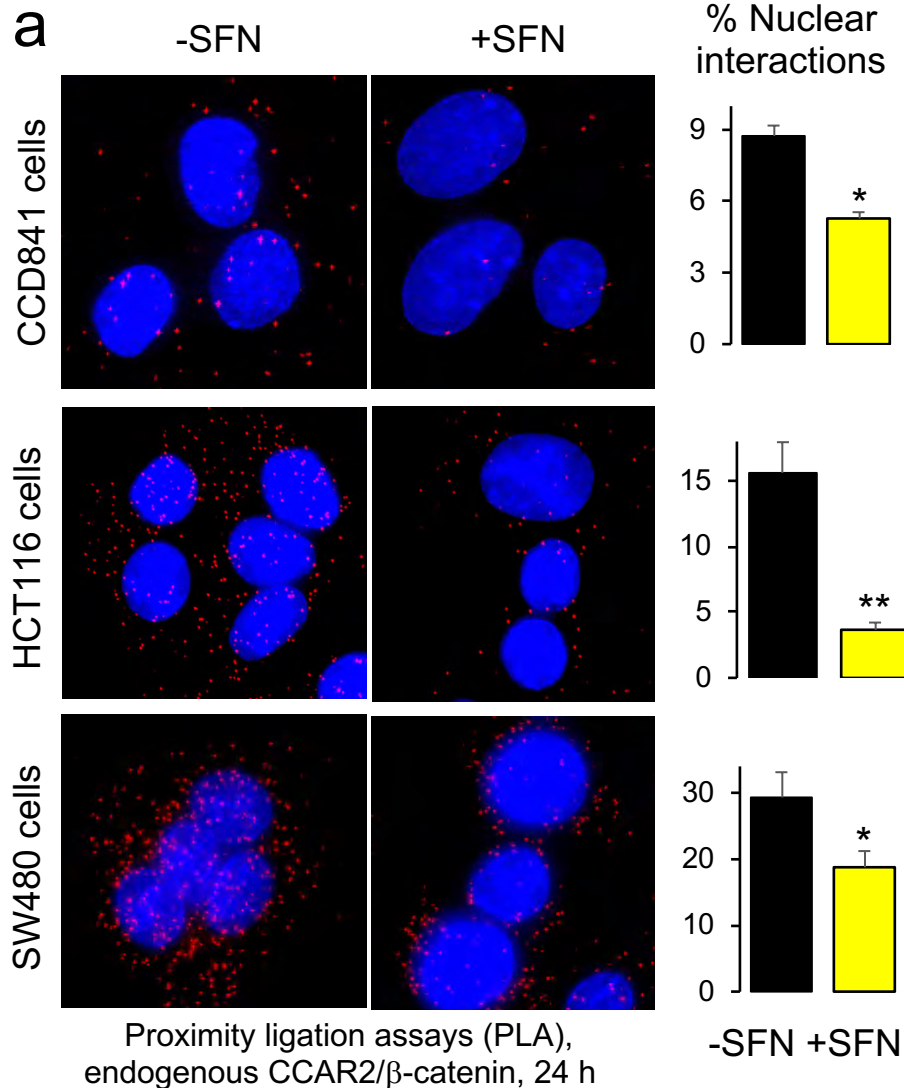


Reduced activation of Wnt/ β -catenin target genes



- MMP7
 - MFAP2
 - FAM60A
 - GAL
 - BMP4
 - EFNB1
 - PRKCG
 - PPP1R1A
 - MET
 - MYC
 - ENPP1
 - MAPRE2
 - TNC
 - PLA2G10
 - CCND1
 - TSPAN5
 - FXYD3
 - MMP9
 - CD24
 - KRTAP3-1
 - DAB2
 - FSCN1
 - SERPINA5
 - IFIH1
 - HSD3B7
 - SEMA3C
 - LGR5
 - AOAH
 - CRMP1
 - EFNB1
 - MSX2
 - GEACAM1
 - CLDN1
 - RAC2
 - SLC7A2
 - SGK1
 - JAG1
 - CKMT2
 - PLAU
 - CXCL1
 - KITLG
 - INHBB
 - AIM1
 - FGF9
 - ABCA1
 - DKK1
 - MYB
 - ENC1
 - CXXC4
 - LRG1
- ← MMP7
- ← MYC
- ← CCND1

CCAR2/ β -catenin interactions are reduced by SFN

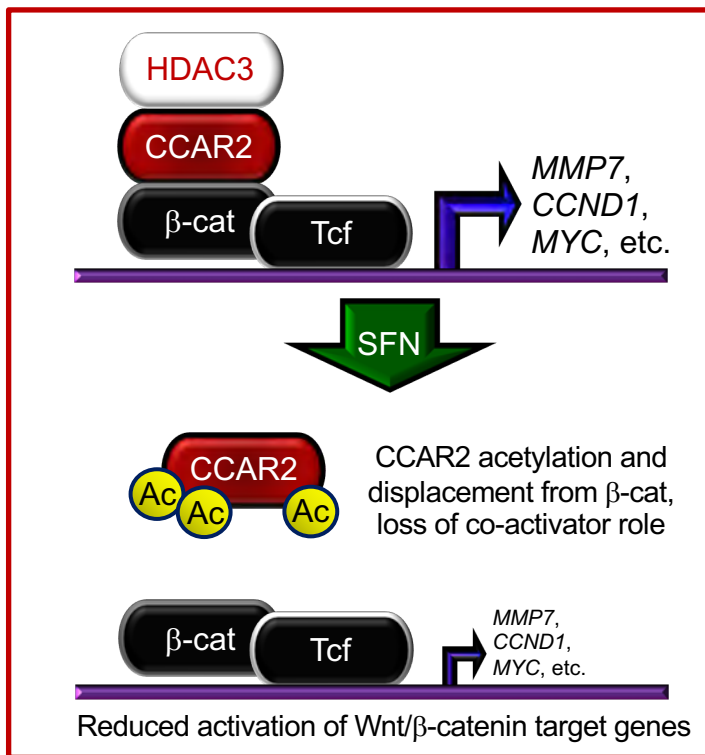


CCAR2/ β -catenin interactions are reduced in humans

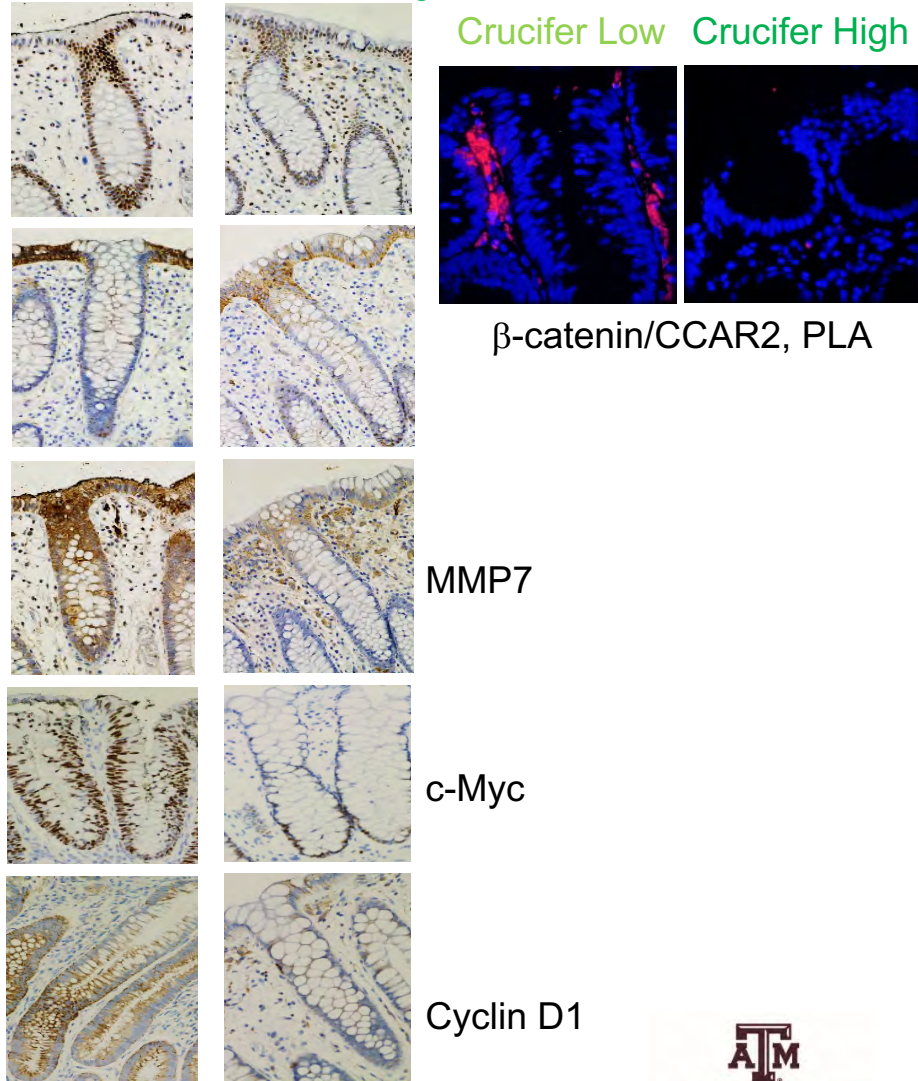
a

Study population
 Age > 50 years
 >5 crucifer servings/week (High)
 0-1 crucifer servings/week (Low)

Phone Screening Cruciferous Vegetable Questionnaire Clinic visit Blood draw Colonoscopy



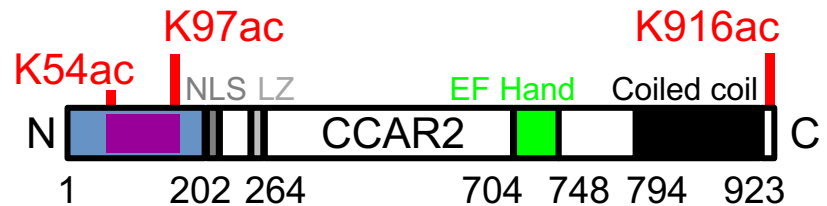
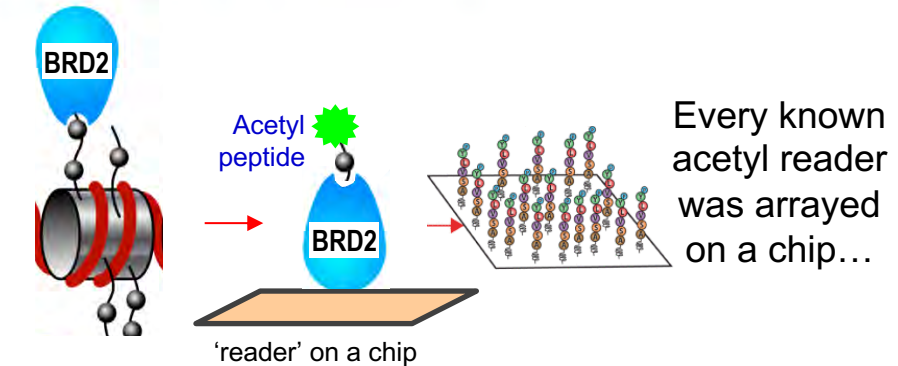
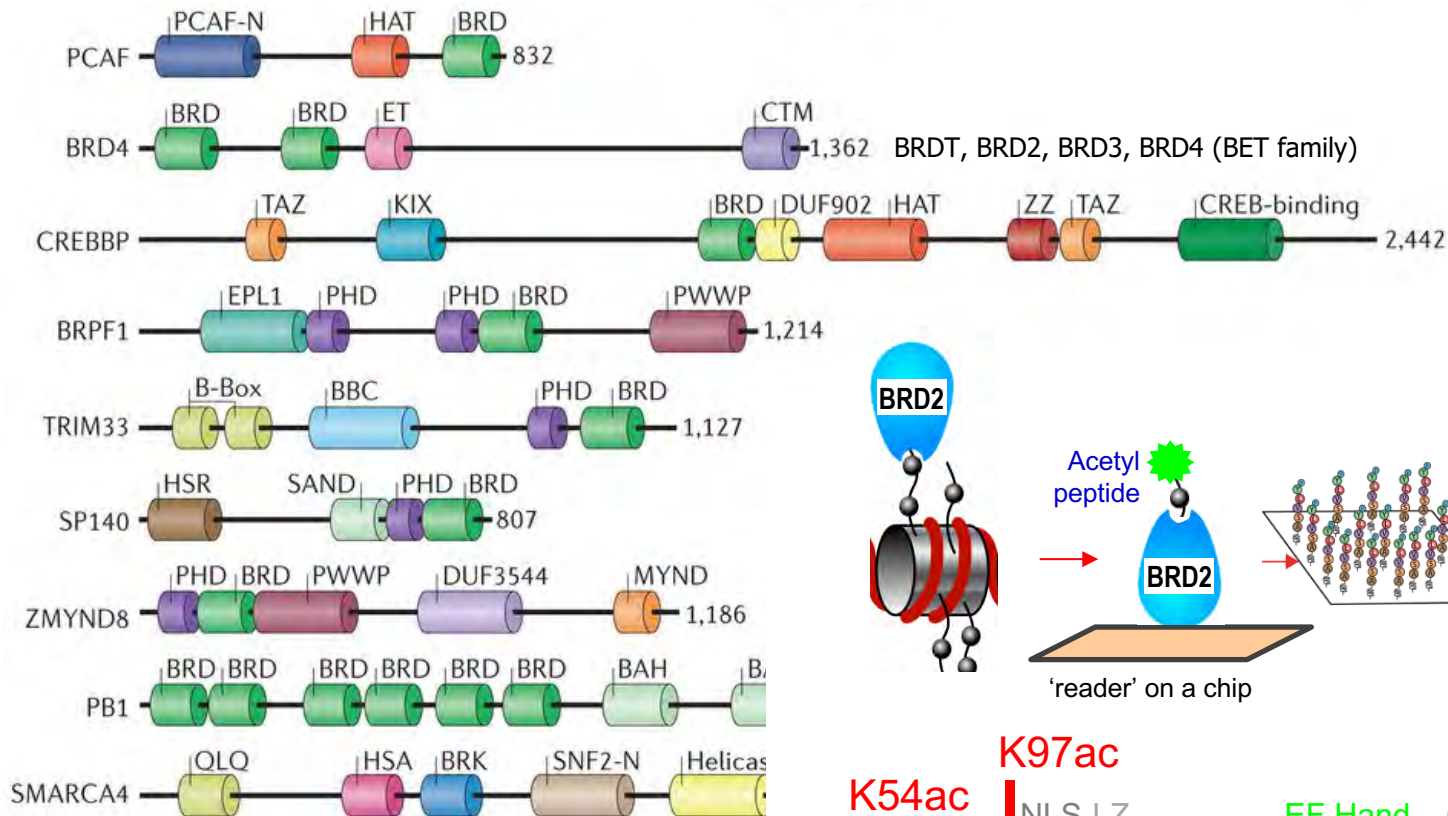
c Crucifer Low Crucifer High



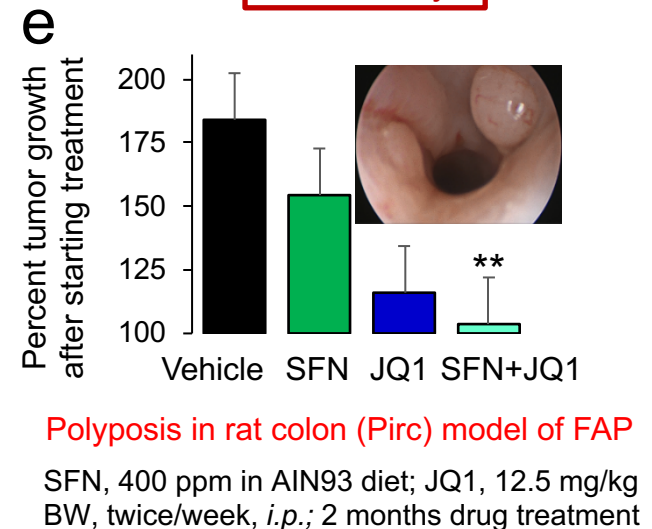
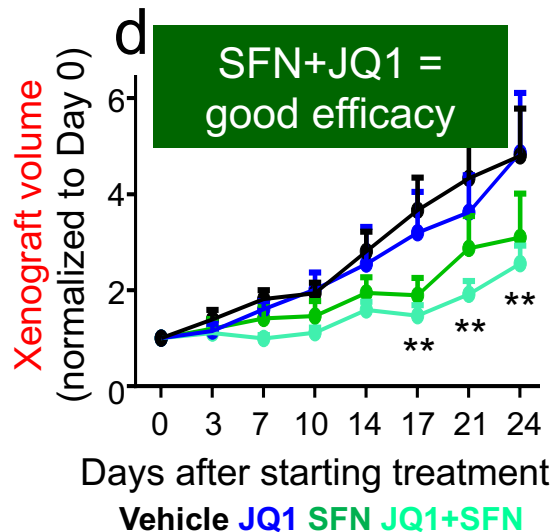
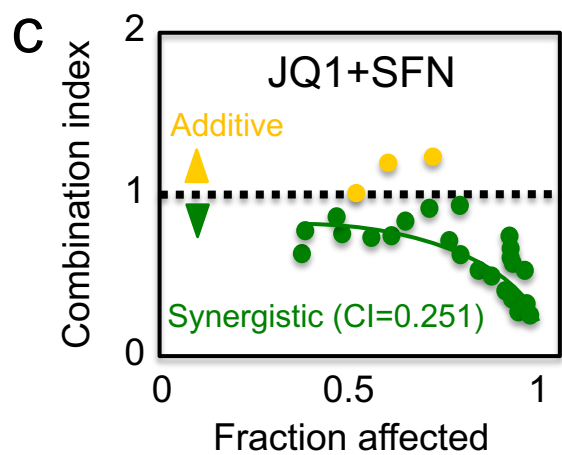
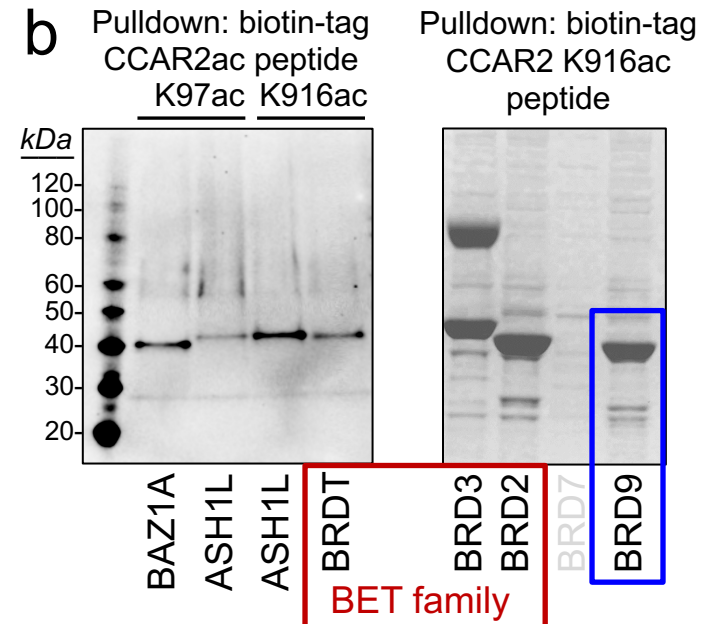
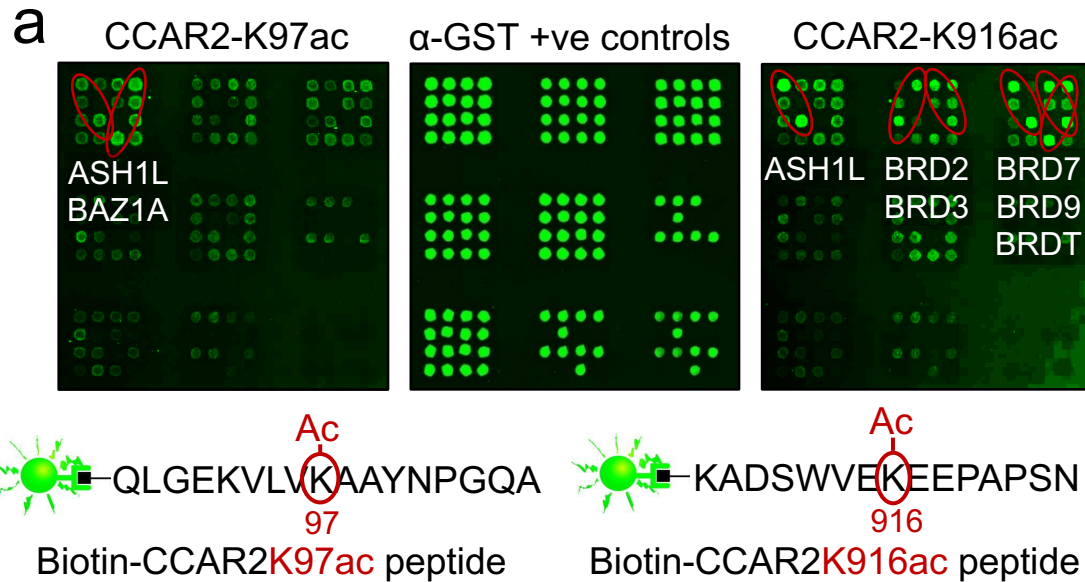
Adenomatous polyps

'Readers' of acetylated proteins

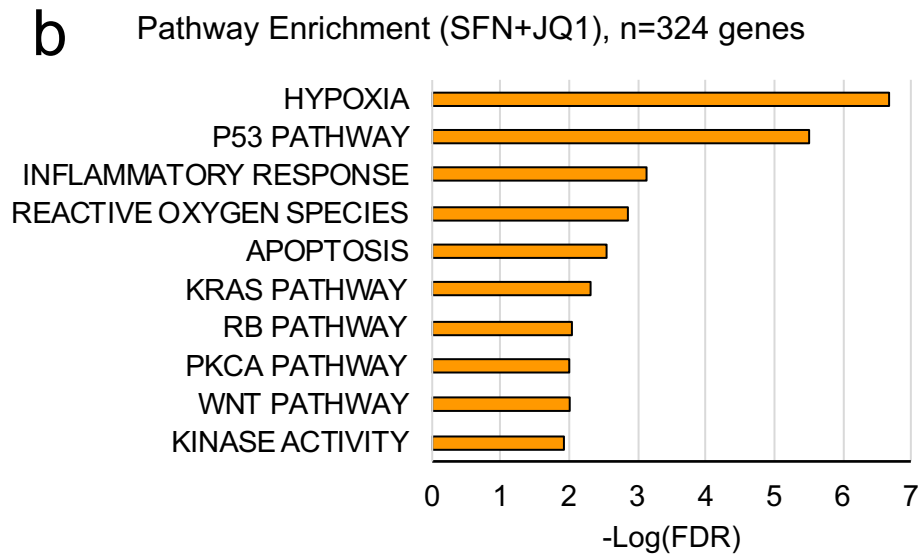
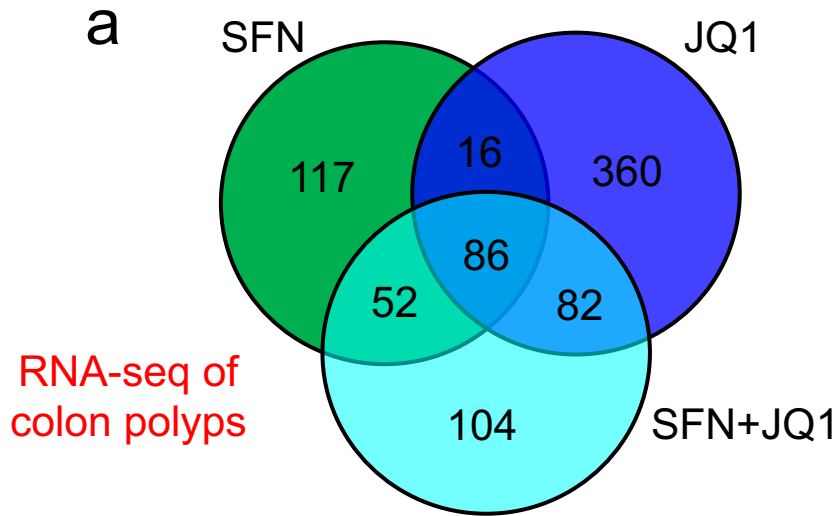
'Functions of bromodomain-containing proteins and their roles in homeostasis and cancer' – Fujisawa & Filippakopoulos 2017



Identifying 'readers' of acetylated CCAR2

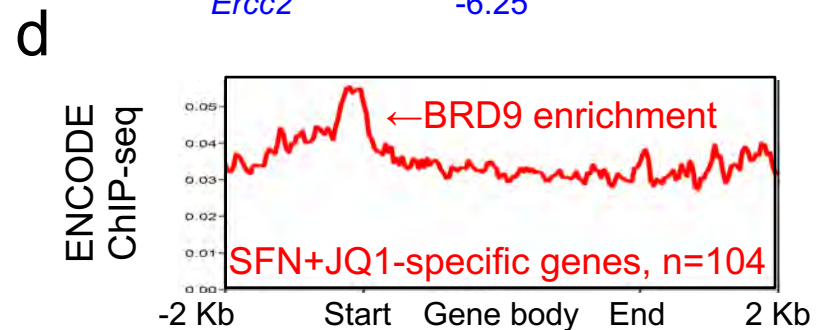


SFN+JQ1: BRD9-regulated targets are implicated

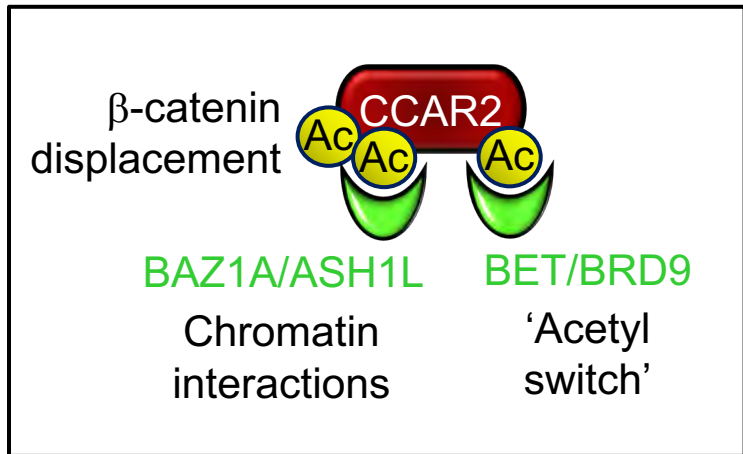
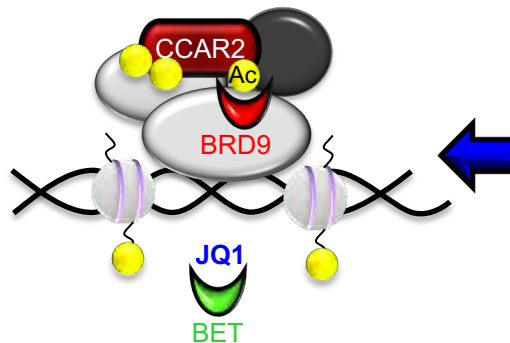
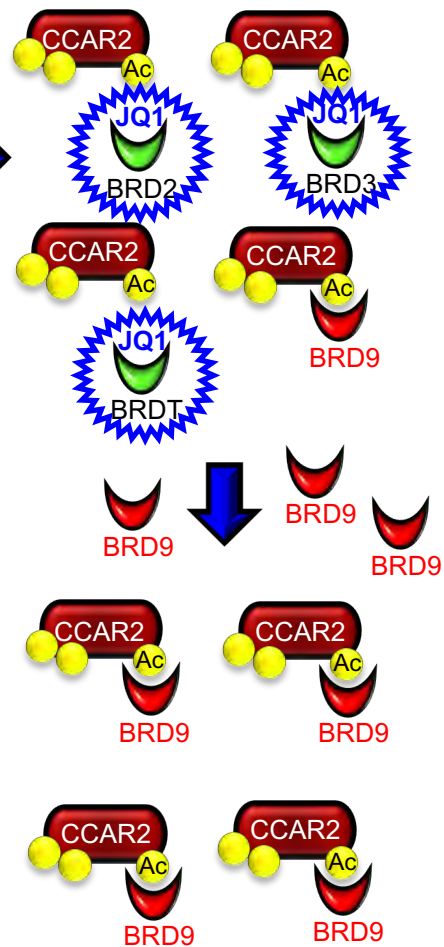
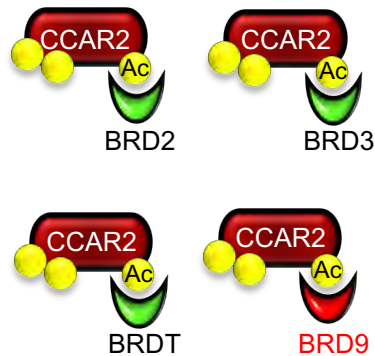


c SFN+JQ1-specific genes

Gene	Fold-change	Regulation
<i>Misp3</i>	3.95	Upregulated
<i>Krt39</i>	3.19	
<i>Anxa13</i>	2.76	
<i>Apobec2</i>	2.63	
<i>Sorcs1</i>	2.52	
<i>Igfbp1</i>	-2.54	Downregulated
<i>Lix1</i>	-2.54	
<i>Mettl7b</i>	-2.55	
<i>Tmem14a</i>	-2.64	
<i>Csf3</i>	-2.71	
<i>Chga</i>	-2.85	
<i>Klk8</i>	-3.08	
<i>Tnnl2</i>	-3.38	
<i>Shh</i>	-3.69	
<i>Reg3b</i>	-3.90	
<i>Tubal3</i>	-4.14	
<i>Prl2a1</i>	-5.56	
<i>Ercc2</i>	-6.25	



Working model for SFN+JQ1



Summary



Prior to SFN-mediated histone acetylation, CCAR2 was acetylated at K54/K97/K916 sites, which **interfered with protein-protein interactions** involving HDAC3 and β -catenin.



Altered β -catenin/CCAR2 interactions and subcellular localization **interfered with the Wnt co-activator role of CCAR2**.



Loss of CCAR2/ β -catenin interactions and **downregulation of β -catenin/Tcf targets** were implicated in a screening colonoscopy trial, in human subjects reporting high vs. low intake of cruciferous vegetables – a surrogate for SFN intake and deacetylase inhibition.



'Readers' of acetyl CCAR2 were identified using protein domain arrays, and included BET members BRD2/BRD3 (known targets of JQ1), and BRD9 (which is not inhibited by JQ1).



JQ1+SFN had **enhanced efficacy** in human colon cancer cells, mouse xenografts, and the Pirc model of FAP, with evidence for downregulation of Wnt/ β -catenin signaling.



A working model for JQ1+SFN proposes **competition between acetyl 'readers'**, and a shift towards increased BRD9-mediated chromatin interactions and target genes.

Acknowledgements



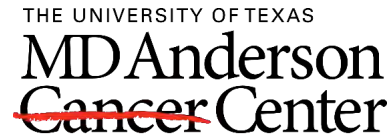
Emily Ho
Christiane Löhrl
Melinda Myzak
David E. Williams
William H. Bisson
P. Andrew Karplus
Barbara Delage
Laura M. Beaver



David A. Lieberman, M.D.



Furkan Ertem
Ahmet Ulsan
Mohaiza Dashwood
Gavin Johnson
Ying-Shiuan Chen
Lindsey Chew
Ahsan Khan
Praveen Rajendran
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Questions?



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